(2)

AD-A197 373

DOCUMENTATION PAGE

Form Approved OMB No 0704-0188

1a REPORT SECURITY CLASSIFICATION: (U)	1b RESTRICTIVE MARKINGS: N/A
2a SECURITY CLASSIFICATION AUTHORITY: N/A	3 DISTRIBUTION / AVAILABILITY OF REPORT: Distribution Unlimited
2b DECLASSIFICATION / DOWNGRADING SCHEDULE: N/A	
4 PERFORMING ORGANIZATION REPORT NUMBER(S): Virginia Commonwealth University	5 MONITORING ORGANIZATION REPORT NUMBER(S): N/A
6a NAME OF PERFORMING ORGANIZATION: Virginia Commonwealth University / 6b OFFICE SYMBOL (If applicable): N/A	7a NAME OF MONITORING ORGANIZATION: Office of Naval Research
6c ADDRESS (City, State, and ZIP Code): 1101 E. Marshall Street, Box 678 MCV Station, Richmond, VA 23298-0678	7b ADDRESS (City, State, and ZIP Code): 800 N. Quincy Street, Arlington, VA 22217-5000
8a NAME OF FUNDING / SPONSORING ORGANIZATION: Office of Naval Research / 8b OFFICE SYMBOL (If applicable): ONR	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: N00014-86-K-0496
8c ADDRESS (City, State, and ZIP Code): 800 N. Quincy Street, Arlington, VA 22217-5000	10 SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO: 61153N / PROJECT NO: RR04108 / TASK NO: / WORK UNIT ACCESSION NO:

11 TITLE (Include Security Classification) (U)
Effects of oligo-PGB On Inflammation and Infectious Diseases.

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13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Final	FROM 8/1986 TO 6/1987	1988, June 28	

16 SUPPLEMENTARY NOTATION

17 COSATI CODES			18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
08			oligo-PGB; Inflammation; Infectious
			Prostaglandins

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

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20 DISTRIBUTION / AVAILABILITY OF ABSTRACT	21 ABSTRACT SECURITY CLASSIFICATION	
☑ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	(U)	
22a NAME OF RESPONSIBLE INDIVIDUAL: Dr. J.A. Majde	22b TELEPHONE (Include Area Code): 202/696-4055	22c OFFICE SYMBOL: ONR

DD Form 1473, JUN 86 — Previous editions are obsolete — SECURITY CLASSIFICATION OF THIS PAGE

S/N 0102-LF-014-6603

88 7 08 04

(U)

SECURITY CLASSIFICATION OF THIS PAGE

June 28, 1988

FINAL REPORT ON CONTRACT N00014-86-K-0496

CONTRACTOR: VIRGINIA COMMONWEALTH UNIVERSITY, SCHOOL OF BASIC HEALTH SCIENCES.

CONTRACT TITLE: EFFECTS OF oligo-PGB ON INFLAMMATION AND INFECTIOUS DISEASES.

START DATE: SEP 01, 1986

RESEARCH OBJECTIVES: To examine the anti - inflammatory effects of oligo-PGB and explore the possible modes of action and mechanisms in order to understand its anti inflammatory effects.

Progress [09/01/86- 07/31/87]

As we stated in the objective, it was paramount to establish whether oligo-PGB functioned as an anti - inflammatory agent in vivo. Therefore, several models of inflammation, in different animal species as well as the effects of administration of oligo-PGB by different routes were tested. A summary of the in vivo anti - inflammatory effects of oligo-PGB in two different species follows.

In the first series of experiments with the C57BL\6J inbred mice, Carrageenin (20 µl of 20 mg/ml solution) was injected into the mouse foot pad, the second foot pad was injected with an equal volume of the vehicle only (0.9% PBS + 5% NaHCO3). The net inflammatory effects of Carrageenin was determined as the total inflammatory response minus the inflammatory effects of the vehicle. The net effects of oligo-PGB (1.35 µg/20 µl dose - 0.5 µg/10 g body weight) on Carrageenin inflammation was determined from the inflammatory response following the simultaneous injection of Carrageenin and oligo-PGB minus the inflammatory response caused by injection of oligo-PGB and vehicle alone. The percent of inflammatory reduction was then calculated from these net values. Two independent methods for measuring inflammation and its reduction were used. The first was based on foot pad thickness at 1, 3 and 5 hours after injection of Carrageenin. The Dyer O.D. reader model, a spring loaded dial calliper was used to obtain the measurements, each value was based on an average of 4 measurements. The second approach was based on determination of the amount of extravasation 5 hours after injection of the irritant in the inflammation site. Radioactive I ^{125} albumin (20 µCi) was injected I.P. one hour prior to tests, and the amount of radioactivity in the foot pad is used as a measurement of extravasation.

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Table 1 provides an overall summary of the anti - inflammatory effects of oligo-PGB in the mouse.

Table 1 : SUMMARY TABLE

ANTI-INFLAMMATORY EFFECT OF oligo-PGB IN VIVO

	Inflammation (mm)			Extravasation (cpm)
AVERAGE CARRAGEENIN EFFECT	0.31	0.34	0.34	28408
AVERAGE oligo-PGB EFFECT	0.21	0.28	0.26	15524
AVERAGE OF CARRAGEENIN /oligo-PGB RATIO	1.66	1.45	4.22	2.94
PERCENT REDUCTION OF INFLAMMATION	0.28	0.13	0.35	0.48

Based on 5 successful experiments, 40 animals were used and 80 treatments done. All foot pads were measured in quadruplicates. All cpm counts were done in duplicates.

In the second series of experiments, the irritant injected into the mouse foot pad was a highly purified human and/or snake PLA_2s. The anti - inflammatory effects of oligo-PGB in this model system are presented ~~in table 2.~~ [illegible] Phospholipase A2, Snake Venom, Synovial fluids Inflammation reduction (Au)

Table 2

Effects of oligo-PGB on Snake Phospholipase A_2 (Naja naja) and on Purified Human Synovial Fluid PLA_2 Inflammation.

	EDEMA (% of Control Sham Injected weight).	
Snake Venom alone	158 ± 3	(n = 4)
Snake Venom + oligo-PGB 20 μM	137 ± 2	"
Snake Venom + oligo-PGB 50 μM	128 ± 2	"
Human Synovial Fluid PLA_2 alone	149 ± 2	"
Human Synovial Fluid PLA_2 + oligo-PGB 20 μM	127 ± 5	"
oligo-PGB alone 50 μM	112 ± 1	"

Saline alone (range 104-108%)

A-1

The route of oligo-PGB administration was examined by testing whether orally administered oligo-PGB would be effective in reducing inflammation in the mouse paw edema model using purified PLA_2s as the irritants. Similarly, the effects of oligo-PGB in the rat carrageenin model was also tested. Results are presented in table 3 and table 4.

Table 3

Effect of oligo-PGB on Phospholipase A_2 - Induced Edema in Mouse Paw Model

MICE (n = 6)	% Increased Edema	% Protection
1. saline control 200 μl	45.7 ± 2.1 (n=6)	0
2. 10 mg/kg oligo-PGB " oral:	35.8 ± 3.5 "	22 %
3. 100 mg/kg oligo-PGB " intubation	17.7 ± 2.0 "	61 %

Table 4

Anti-Inflammatory Activity of oligo-PGB in Carrageenin-Induced Rat Paw Edema

	Foot Width (mm)	% of Control
Control	4.2 (n=10)	100%
Control + carrageenin	8.7 (n=8)	207%
Control + carrageenin + oligo-PGB*	6.4 (n=5)	152%

* oligo-PGB (1.5 mg/kg) injected IP 1 hr prior to irritant

These results clearly establish the anti - inflammatory effects of oligo-PGB. Most importantly these data show the effectiveness of oligo-PGB administered by the oral route.

Our previous studies had demonstrated conclusively that oligo-PGB 29-II inhibited human, non pancreatic, Ca2+-dependent phospholipases A2 (PLA_2s) active at neutral pH, and the inositol-specific phospholipase C. The PLA_2 isolated from various human sources (including neutrophils, platelets, plasma, synovial fluid) was approximately an order of magnitude more sensitive to inhibition by oligo-PGB; IC_50 = 4-8 μM, PLA_2 vs 50-100 μM, PLC. In addition we were able to show that oligo-PGB had potent anti-oxidant activity (figure 1) using air exposure of phospholipid as a model of auto-oxidation. The anti-PLA_2 and anti-oxidant properties of oligo-PGB in vitro indicated that these polymers may be cytoprotective, could influence the arachidonic acid cascade, and therefore could function as anti-inflammatory agents.

Studies with prelabelled human endothelial cells in culture and prelabelled human neutrophils have subsequently shown that oligo-PGB inhibits ionophore (and histamine) stimulated mobilization of arachidonic acid from membrane phospholipid (table 5); and this is reflected by not only depressed levels of unesterified arachidonate but also decreased levels of prostaglandins and leukotrienes in general (not shown).

Table 5

Inhibition of Arachidonate Mobilization by oligo-PGB Using Prelabelled Human Neutrophils

Preincubation oligo-PGB	Albumin	Control (C)	Percent inhibition	A23187	C - S (S)	Percent inhibition
0	5 μM	3,518	-	10,668	7,150	-
10 μM	5 μM	2,459	30.1%	5,598	3,139	56.1%
20 μM	10 μM	2,301	34.6%	4,721	2,420	66.2%
30 μM	15 μM	2,692	23.5%	5,399	2,707	62.1%
40 μM	20 μM	2,647	25.3%	4,374	1,746	75.6%
10 μM	20 μM	3,109	11.6%	11,968	8,859	no effect

SUMMARY:

The results have establish in-vivo the anti - inflammatory effects of oligo-PGB. The biochemical data, provide a fundamental understanding of how oligo-PGB can protect various cells and organ systems from injury by focusing on biochemical events at the level of the cell membrane. The in vitro studies illustrating anti-lipolytic and anti-oxidant activities of oligo-PGB, and the confirmation of these effects on cellular signal and transduction events as measured by depressed arachidonate mobilization and prostanoid formation provide a molecular basis for the anti-inflammatory and cytoprotective action of oligo-PGB.

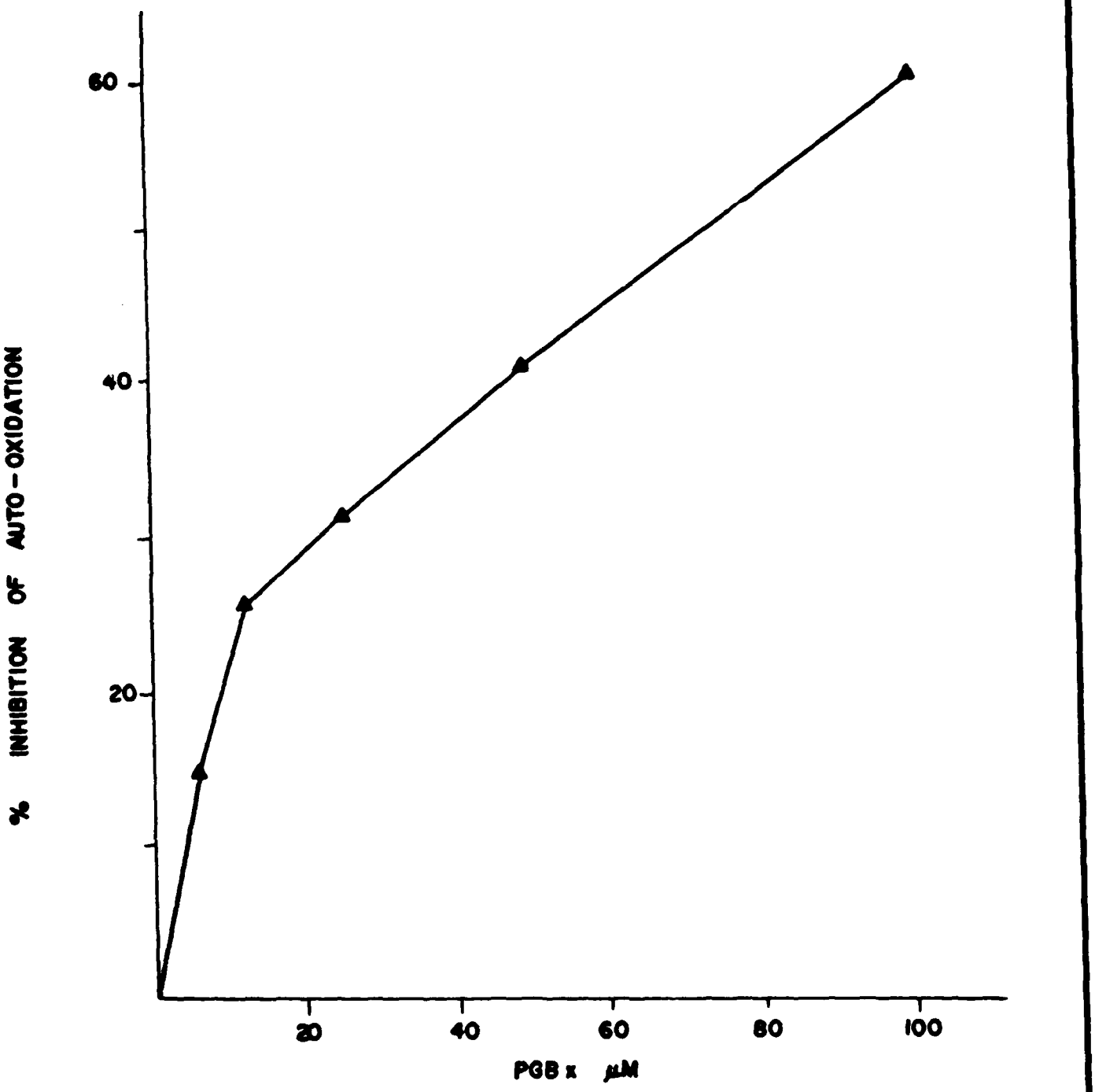

Figure 1 Inhibition of Auto-Oxidation of Phosphatidylethanolamine by PGBx